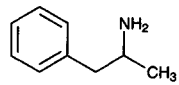
Amphetamine



Molecular formula: $C_9H_{13}N$
Molecular weight: 135.21
CAS Registry No.: 300-62-9, 139-10-6 (phosphate), 60-13-9 (sulfate), 1407-85-8 (d-form tannate)
Merck Index: 623
Lednicer No.: 1 37, 70; 2 47

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 µL 100 µg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 µL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES

Column: 250 × 4.6 7 µm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM

Retention time: 8.0 (R-(-)), 9.5 (S-(+))

OTHER SUBSTANCES

Extracted: methamphetamine, tranylcypromine

KEY WORDS

plasma; derivatization; normal phase; chiral

REFERENCE

Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE

Matrix: blood
Sample preparation: 100 µL Serum + 50 µL 100 ng/mL aniline sulfate in water + 200 µL 20 mM pH 10.6 carbonate buffer + 2 mL ethyl acetate, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the organic layer and add it to 200 µL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the aqueous layer and add it to 40 µL 250 mM NaOH, add 50 µL 330 mM pH 7.8 phosphate buffer, add 250 µL MeCN, add 25 µL 1 mM (-)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand overnight at room temperature, add 30 µL 100 mM glycine in water, add 750 µL n-pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in MeCN:water 50:50, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: Direct-Connect column prefilter (Alltech)
Column: 150 × 4.6 3 µm Adsorbosphere HS C18 (Alltech)

Mobile phase: MeCN:THF:20 mM pH 3.6 acetate buffer 39:15:46
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 22.6 (R), 23.6 (S)
Internal standard: aniline (21.0)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
serum; rat; chiral; derivatization

REFERENCE
Hutchaleelaha,A.; Walters,A.; Chow,H.-H.; Mayersohn,M. Sensitive enantiomer-specific high-performance liquid chromatographic analysis of methamphetamine and amphetamine from serum using precolumn fluorescent derivatization, *J.Chromatogr.B*, **1994**, *658*, 103–112.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 11 borate buffer, mix, add 2.5 mL diethyl ether, vortex for 5 min, centrifuge at 1200 g for 5 min, remove organic layer, repeat extraction. Combine the organic layers and add them to 200 μL 100 mM HCl, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 150 μL 1 M pH 8 borate buffer and 100 μL 4 mM 9-fluorenylmethyl chloroformate in MeCN, shake, allow to react at 50° for 5 min, add 20 μL 20 mM proline in water, allow to react at 50° for 2 min, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 50:50
Flow rate: 1
Injection volume: 200
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine, desmethyldeprenyl

KEY WORDS
plasma

REFERENCE
La Croix,R.; Pianezzola,E.; Strolin Benedetti,M. Sensitive high-performance liquid chromatographic method for the determination of the three main metabolites of selegiline (L-deprenyl) in human plasma, *J.Chromatogr.B*, **1994**, *656*, 251–258.

SAMPLE
Matrix: blood, tissue, dialysate
Sample preparation: Plasma. 45 μL Plasma + 5 μL 40 μM tryptamine + 100 μL 100 mM borate buffer adjusted to pH 10.6 with NaOH + 200 μL ethyl acetate, vortex for 2 min,

let sit on ice for 10 min, add 200 μL ethyl acetate, add 200 μL water, vortex briefly, centrifuge at 4° at 18000 g for 10 min. Remove 200 μL of the organic supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 80 μL 50 mM KH_2PO_4 adjusted to pH 2.6 with phosphoric acid, add 20 μL borate buffer adjusted to pH 11.5 with NaOH, add 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. Tissue. Sonicate brain tissue with 9 volumes of 8 μM tryptamine in 100 mM pH 10.6 borate buffer for 20 s, centrifuge at 4° at 18000 g for 10 min, remove a 100 μL aliquot of the supernatant and add 200 μL ethyl acetate, vortex for 2 min, let sit on ice for 10 min, add 200 μL ethyl acetate, add 200 μL water, vortex briefly, centrifuge at 4° at 18000 g for 10 min. Remove 200 μL of the organic supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 80 μL 50 mM KH_2PO_4 adjusted to pH 2.6 with phosphoric acid, add 20 μL borate buffer adjusted to pH 11.5 with NaOH, add 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. Dialysate. 100 μL Dialysate + 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. (Reagent was 27 mg o-phthaldialdehyde in 500 μL EtOH, add 5 mL 100 mM pH 9.6 borate buffer, add 40 μL 3-mercaptopropionic acid, refrigerate, use for up to 4 days.)

HPLC VARIABLES

Guard column: 20 × 2 37-50 μm Bondapak C18/Corasil
Column: 250 × 4.6 5 μm LC-18 (Supelco)
Mobile phase: Gradient. MeOH:buffer 35:65 for 3 min, to 65:35 over 1 min, maintain at 65:35 for 14 min, return to initial conditions over 1 min, re-equilibrate for 6 min. (Buffer was 50 mM KH_2PO_4 adjusted to pH 5.5 with KOH.)
Flow rate: 1.5
Injection volume: 75
Detector: F ex 340 em 440

CHROMATOGRAM

Retention time: 14.5
Internal standard: tryptamine (13)
Limit of detection: 200 pg (plasma, tissue), 50 pg (dialysate)
Limit of quantitation: 100 pg (dialysate)

OTHER SUBSTANCES

Extracted: metabolites, p-hydroxyamphetamine

KEY WORDS

rat; brain; plasma; derivatization

REFERENCE

Bowyer,J.F.; Clausing,P.; Newport,G.D. Determination of d-amphetamine in biological samples using high-performance liquid chromatography after precolumn derivatization with o-phthaldialdehyde and 3-mercaptopropionic acid, *J.Chromatogr.B*, **1995**, *666*, 241–250.

SAMPLE

Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL 8% sodium bicarbonate, add 1 mL 0.5% sodium 1,2-naphthoquinone-4-sulfonate, heat at 70° for 20 min, add an equal volume of chloroform, vortex for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES

Column: 150 × 5 Partisil 5
Mobile phase: Hexane:chloroform:ethyl acetate:EtOH 50:25:35:1

Column temperature: 20
Flow rate: 2.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 5
Internal standard: phenylethylamine (6)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: hydroxyamphetamine, methamphetamine, norephedrine

KEY WORDS
derivatization; plasma; normal phase; comparison with other derivatization reagents and with ion-pair chromatography; SPE

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL EtOH, add 1 mL reagent, mix, filter, inject a 50 μL aliquot. (Prepare reagent by dissolving 200 mg o-phthalaldehyde in 2 mL MeOH, add 400 μL mercaptoethanol, add to buffer, store in the dark in the refrigerator, discard after 5 days. Prepare buffer by dissolving 1 g boric acid in 38 mL water and adjusting pH to 10.4 with 4 M KOH.)

HPLC VARIABLES
Column: 200 × 5 10 μm Partisil ODS-2
Mobile phase: MeOH:water 73:27 containing 0.2% EDTA
Column temperature: 20
Flow rate: 1.8
Injection volume: 50
Detector: F ex 345 em 445

CHROMATOGRAM
Retention time: 9
Internal standard: benzylamine (6)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: hydroxyamphetamine, norephedrine

KEY WORDS
derivatization; plasma; comparison with other derivatization reagents and with ion-pair chromatography; SPE

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 1 mL 120 mM sodium dodecyl sulfate in 100 mM NaOH, homogenize, filter (45 μm). Inject on to column A at 180 μL/min 200 μL 60 mM sodium dodecyl sulfate in MeCN:water 50:50, 25 μL filtrate, and 25 μL 60 mM sodium dodecyl sulfate in MeCN:water 10:90, wait for 1 min, inject 100 μL 60 mM sodium dodecyl sulfate in MeCN:water 10:90, inject 200 μL 10 mM sodium dodecyl sulfate in MeCN:water 10:90, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 300 μL 60 mM sodium dodecyl sulfate in MeCN:water 50:50 and 300 μL 10 mM sodium dodecyl sulfate in MeCN:water 80:20. Urine. 5 mL Urine + 4.5 mL MeCN + 500 μL 1 M KOH, centrifuge at 2500 rpm for 10 min, filter (45 μm) the supernatant. Inject on to column A at 180 μL/min 25 μL 50 mM KOH in MeCN:water 20:80, 50 μL filtrate, 25 μL 50 mM KOH in MeCN:water 20:80, and 200 μL MeCN:water 20:80, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 400 μL MeCN.

HPLC VARIABLES

Column: A 20 × 2 polymeric reagent; B 250 × 4.6 5 μm Supelcosil LC-18-DB (with a guard column) (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

Mobile phase: Gradient. MeCN:water 50:50 for 3.5 min, to 70:30 over 12 min, maintain at 70:30 for 2.5 min, return to initial conditions over 1 min. (Place a 100 × 4.6 30-40 μm silica column before the injector.)

Column temperature: 60 (column A only)

Injection volume: 25-50

Detector: F ex 254 em 305-395

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 25 ng/mL (urine), 600 ng/mL (serum)

OTHER SUBSTANCES
Extracted: methamphetamine (only in urine)

KEY WORDS
derivatization; serum

REFERENCE
Bourque,A.J.; Krull,I.S.; Feibush,B. Automated HPLC analyses of drugs of abuse via direct injection of biological fluids followed by simultaneous solid-phase extraction and derivatization with fluorescence detection, *Biomed.Chromatogr.*, **1994**, *8*, 53–62.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.71

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Simultaneous: ephedrine, methamphetamine, phenmetrazine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: bulk
Sample preparation: Mix 200 μmole amine with 500 μmole (1S)-(+)-camphor-10-sulfonyl chloride, 10 mL diethyl ether, and 10 mL 1 M NaOH, stir vigorously for 1 h, acidify with concentrated HCl, extract three times with diethyl ether. Combine the organic layers and wash them three times with water, evaporate to dryness, reconstitute with 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Silica 100-RP 18
Mobile phase: MeOH:water 50:50
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 15.55, 18.13 (enantiomers)

OTHER SUBSTANCES
Also analyzed: bamethan, ephedrine, norpseudoephedrine, 1-phenylethylamine

KEY WORDS
derivatization; chiral

REFERENCE
Vogt,C.; Jira,T.; Beyrich,T. HPLC-Trennung racemischer Amine nach Derivatisierung mit (1S)-(+)-Campher-10-sulfonylchlorid, *Pharmazie*, **1990**, *45*, 691.

SAMPLE
Matrix: bulk
Sample preparation: Reflux a 10% excess of reagent in toluene for 10 min, add the drug, let stand at room temperature for 10 min, cool, dilute, inject an aliquot. (The reagent was N-(p-toluenesulfonyl)prolyl azide and was prepared as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles p-toluenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above

for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(p-toluene)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!) (Anal.Chem. 1984, 56, 958). Suspend 86 mmoles 1-[(p-toluene)sulfonyl]proline in 15 mL water and add sufficient acetone to give a clear solution, cool to 0°, add 10.2 g triethylamine in 175 mL acetone, slowly add 12.5 g ethyl chloroformate in 45 mL acetone while maintaining the temperature at 0°, stir at 0° for 30 min, add dropwise 8.6 g sodium azide in 30 mL water, stir at 0° for 1 h, pour into ice water, extract with ether, dry over anhydrous magnesium sulfate, evaporate under reduced pressure at room temperature to give N-(p-toluenesulfonyl)prolyl azide (cf J.Org.Chem. 1961, 26, 3511).)

HPLC VARIABLES
Column: 300 × 4 7-9 μm silica gel
Mobile phase: Petroleum ether:isopropanol 96.5:3.5
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 13, 16 (enantiomers)

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Zhou,Y.; Sun,Z.P.; Lin,D.K. Liquid chromatographic evaluation of a new chiral derivatizing agent for enantiomeric resolution of amine and alcohol drugs, *J.Liq.Chromatogr.*, **1990**, *13*, 875–885.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 11.86, k' 12.48 (enantiomers)

OTHER SUBSTANCES
Simultaneous: normetoprolol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: bulk, formulations
Sample preparation: 10 mg Bulk drug, tablet, or capsule or 10 mL 1 mg/mL syrup + 5 mL 20% NaOH, sonicate until dissolved, add 10 mL 10 mM 2 naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with two 5 mL portions of 10 mM sulfuric acid, filter through a cotton plug, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 35-50 μm silica
Column: 250 × 4.6 Pirkle Covalent Phenylglycine (Regis)
Mobile phase: Hexane:isopropanol:MeCN 97:3:0.5
Column temperature: 20
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 20 (-), k' 22 (+)

KEY WORDS
tablets; capsules; syrup; chiral; derivatization; 1% of minor enantiomer can be detected

REFERENCE
Wainer,I.W.; Doyle,T.D.; Adams,W.M. Liquid chromatographic chiral stationary phases in pharmaceutical analysis: determination of trace amounts of the (-)-enantiomer in (+)-amphetamine, *J.Pharm.Sci.*, **1984**, *73*, 1162–1164.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dissolve 10 mg bulk drug in 5 mL dichloromethane, add 5 mL 20% NaOH, add 10 mL 10 mM 2-naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with 5 mL 10 mM sulfuric acid. Filter the organic layer through a syringe containing a glass wool plug and anhydrous sodium sulfate, inject a 10 μL aliquot. Capsules. Sonicate 1 capsule in 5 mL 20% NaOH for 45 min or until dissolved, filter, add 5 mL dichloromethane to filtrate, add 10 mL 10 mM 2-naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with 5 mL 10 mM sulfuric acid. Filter the organic layer through a syringe containing a glass wool plug and anhydrous sodium sulfate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Bakerbond Chiral Phase [DNBPG]
Mobile phase: Hexane:isopropanol:MeCN 97:3:0.5
Column temperature: 20

Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 28.5 (l), 31 (d)

KEY WORDS
capsules; chiral; derivatization

REFERENCE
Alembik,M.C.; Wainer,I.W. Resolution and analysis of enantiomers of amphetamines by liquid chromatography on a chiral stationary phase: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 530–533.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM
Retention time: 3.62
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: ephedrine, methamphetamine, caffeine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: meconium
Sample preparation: 500 mg Meconium + 5 mL water + 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Remove the supernatant and add it to 5 mL water and 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Make up the supernatant to 20 mL with pH 9.0 borax buffer, add it to an Extrelut SPE cartridge, after 10 min elute with 60 mL dichloromethane:isopropanol 80:20. Add 1 drop 2% tartaric acid in water and evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18 DB

Mobile phase: MeOH:MeCN:THF:triethylamine:water 100:25:7:1.5:600 containing 77 mM KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 204

CHROMATOGRAM
Retention time: k' 4.3
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: morphine
Noninterfering: caffeine, benzoylecgonine, cocaine, codeine

KEY WORDS
SPE

REFERENCE
Franssen,R.M.E.; Stolk,L.M.L.; van den Brand,W.; Smit,B.J. Analysis of morphine and amphetamine in meconium with immunoassay and HPLC-diode-array detection, *J.Anal.Toxicol.*, **1994**, *18*, 294–295.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL 20-300 μg/mL amine solution in water with 2 mL 50 mg/mL 4-nitrobenzoyl chloride in THF (freshly prepared) and 1 mL 1 M NaOH, heat at 65° for 1 h, cool, adjust pH to 12 with 1 M NaOH, extract with two 10 mL portions of chloroform. Combine the extracts and wash them with two 20 mL portions of 10% potassium carbonate, wash with water, dry over anhydrous magnesium sulfate. Evaporate to dryness under a stream of air, reconstitute the residue in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 35:65
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: benzylamine, methamphetamine, α-methylbenzylamine, n-propylamphetamine

KEY WORDS
derivatization

REFERENCE
Clark,R.C.; Teague,J.D.; Wells,M.M.; Ellis,J.H. Gas and high-pressure liquid chromatographic properties of some 4-nitrobenzamides of amphetamines and related arylalkylamines, *Anal.Chem.*, **1977**, *49*, 912–915.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.62

OTHER SUBSTANCES

Simultaneous: morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL 1 mg/mL Compound in dichloromethane + 50 μL 7.6 mM 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in MeCN or dichloromethane: triethylamine 99.8:0.2, mix, let stand at room temperature for 1 h, add 1 mL 1 M HCl, shake mechanically for 5 min, centrifuge at 1000 g for 15 min, discard the aqueous phase, add 1 mL 1 M NaOH, shake for 5 min, centrifuge at 1000 g for 15 min. Remove a 10 μL aliquot of the organic layer and dilute it to 1 mL with MeOH, inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm octadecyl (IBM)
Mobile phase: MeOH:water 55:45
Flow rate: 1-2
Injection volume: 20-50
Detector: UV 254

CHROMATOGRAM

Retention time: 16.8 (S), 18.2 (R)
Limit of quantitation: 100 ng

OTHER SUBSTANCES

Simultaneous: 1-(4-chlorophenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, 1-(2,4-dimethoxy-5-methylphenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-thiomethylphenyl)-2-aminopropane, 1-phenylethylamine

KEY WORDS
derivatization; comparison with other derivatizing reagents; chiral

REFERENCE
Miller,K.J.; Gal,J.; Ames,M.M. High-performance liquid chromatographic resolution of enantiomers of 1-phenyl-2-aminopropanes (amphetamines) with four chiral reagents, *J.Chromatogr.*, **1984**, *307*, 335–342.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 1 mg/mL Compound in dichloromethane + 100 μL 6.8 mM (R)-(+)-1-phenylethylisocyanate in dichloromethane, mix, let stand at room temperature for 1 h, evaporate to dryness under a stream of nitrogen, add 1 mL 100 mM NaOH, vortex for 15 min, add 1 mL 20% NaOH, add 3 mL dichloromethane, shake mechanically for 15 min, centrifuge at 1000 g for 15 min. Remove the organic layer and wash it with 2 mL 100 mM HCl. Remove a 100 μL aliquot of the organic layer and dilute it to 1 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm octadecyl (IBM)
Mobile phase: MeOH:water 60:40
Flow rate: 1-2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13.5 (R), 14.0 (S)
Limit of quantitation: 100 ng

OTHER SUBSTANCES
Simultaneous: 1-(4-chlorophenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, 1-(2,4-dimethoxy-5-methylphenyl)-2-aminopropane, 1-phenylethylamine

KEY WORDS
derivatization; comparison with other derivatizing reagents; chiral

REFERENCE
Miller,K.J.; Gal,J.; Ames,M.M. High-performance liquid chromatographic resolution of enantiomers of 1-phenyl-2-aminopropanes (amphetamines) with four chiral reagents, *J.Chromatogr.*, **1984**, *307*, 335–342.

SAMPLE
Matrix: solutions
Sample preparation: 2 mL THF + 1 mL 33.5 mM reagent in THF (freshly prepared) + 1 mL 1 mg/mL amphetamine in water + 700 μL 10% sodium bicarbonate in water, heat at 65° for 1 h, cool, extract three times with 10 mL aliquots of chloroform. Combine the extracts and wash them with 10 mL water, dry over anhydrous magnesium sulfate, evaporate to dryness, reconstitute with 2.5 mL mobile phase, inject a 5 μL aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the

reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (Anal.Chem. 1984, 56, 958) (mp 110-110.5°).)

HPLC VARIABLES
Guard column: 70 × 2.1 30-38 μm HC Pellosil (Whatman)
Column: 150 × 4.6 5 μm Supelcosil LC-Si
Mobile phase: n-Heptane:chloroform 20:80
Flow rate: 1.4
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4 (R), 4.5 (S)

KEY WORDS
derivatization; normal phase; chiral

REFERENCE
Barksdale,J.M.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine and related drugs by diastereomeric derivatization, *J.Chromatogr.Sci.*, **1985**, *23*, 176–180.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine, ephedrine, hydroxyamphetamine, methamphetamine, phentermine, mephentermine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 2 mg in 15 mL 0.45 M NaOH, extract twice with 30 mL chloroform. Combine the organic layers and add a 10% molar excess of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in chloroform, let stand for 10 min, evaporate the chloroform under a stream of air, dissolve the residue in 1 mL THF or MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water:acetic acid 50:49:1
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 25 (R), 28 (S)

OTHER SUBSTANCES
Simultaneous: norpseudoephedrine, norephedrine

KEY WORDS
chiral; derivatization

REFERENCE
Noggle,F.T.,Jr.; DeRuiter,J.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine prepared from norephedrine and norpseudoephedrine, *J.Chromatogr.Sci.*, **1987**, *25*, 38–42.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Simultaneous: acetophenone, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

Sample preparation: Mix sample:50 mM (?) NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 65

OTHER SUBSTANCES
Simultaneous: baclofen, tranylcypromine

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: solutions
Sample preparation: Mix 500 μL of a solution in 20 mM pH 9.5 borate buffer with 100 μL 10 mM NaCN in 20 mM pH 9.5 borate buffer, add 500 μL 0.1 mM naphthalene-2,3-dicarboxaldehyde in MeOH, mix, let stand at room temperature for 20 min, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.1 5 μm LiChrosorb RP-18
Mobile phase: MeCN:2.5 mM pH 7.0 imidazole buffer 75:25
Flow rate: 0.5
Injection volume: 25
Detector: Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl)oxalate pumped at 0.2 mL/min and the mixture flowed into the detector.

CHROMATOGRAM
Limit of detection: 4 fmole

KEY WORDS
derivatization

REFERENCE
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

SAMPLE
Matrix: solutions

Sample preparation: Inject a 10 μL aliquot of a 100 ppm solution in MeCN:dichloromethane:triethylamine 50:50:0.05 into the mobile phase. The mobile phase flows through a 27 × 2.2 reactor packed with reagent at 72° to the column. (Reagent was dinitrobenzoyl-o-nitrobenzophenone polymeric reagent, prepared as follows. (Caution! Dioxane is carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) Soxhlet extract 200-400 mesh polystyrene cross-linked with 4% divinylbenzene (Fluka) with MeCN for 48 h. Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to a mixture of 50 g of the polystyrene resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir the mixture mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice. Wash the beads with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm DMF (60°), wash with six 300 mL portions of dichloromethane:MeOH 2:1, dry. Add the polymer to 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane, heat at 90° for 8 h, filter, repeat the process, filter, wash the beads with four portions of warm (60°) dioxane, add 30 mL acetic acid, stir for 15 min, wash with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-(R)-Naphthyl Urea (Supelco)
Mobile phase: Hexane:dichloromethane:THF 70:27:3
Flow rate: 0.1 for 40 s, to 3.1 over 30 s, maintain at 3.1
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: 11.5 (D), 13 (L)

KEY WORDS

derivatization; chiral

REFERENCE

Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 2.220

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:500 mM pH 9.0 borate buffer 50:50 with 25 mg reagent, after 1 min elute with 1 mL hexane:THF 75:25, inject a 5 μL aliquot. (Reagent was dinitrophenyl carbamate benzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Dissolve 3,5-dinitrobenzoyl chloride in the minimum amount of glacial acetic acid, add an equimolar amount of sodium azide, stir for 30 min, dilute with water, filter to obtain 3,5-dinitrobenzoyl azide (Caution! Azides are toxic and potentially explosive!) (J. Liq. Chromatogr. 1986, 9, 443). Heat 71 mg 3,5-dinitrobenzoyl azide in 15 mL toluene (dried over calcium hydride) at ??? for 30 min, cool using an ice bath, add 200 mg polymer, allow to warm to room temperature with stirring for 1 h, filter, wash the polymer with four 10 mL portions of warm (40°) dichloromethane, dry under high vacuum for 1 h.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-(R)-naphthylurea (Supelco)
Mobile phase: Hexane:EtOH:MeCN 93:7:0.5
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 12.2, 15.7 (enantiomers)

OTHER SUBSTANCES

Simultaneous: methamphetamine

KEY WORDS

derivatization; chiral

REFERENCE

Bourque,A.J.; Krull,I.S. Immobilized isocyanates for derivatization of amines for chiral recognition in liquid chromatography with UV detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 495–503.

SAMPLE

Matrix: solutions

Sample preparation: 50 μL 5 mg/mL Amphetamine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 21.8 mg 1-thio-β-D-glucose in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 55:2:45 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 12.37 (R-(-)), 14.12 (S-(+))

KEY WORDS
derivatization; protect from light; chiral

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, methamphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL aliquot of a 0.1-1 mM solution in 10 mM HCl with 500 μL 100 mM pH 9.5 borate buffer, 100 μL 2.5 mM N-acetyl-L-cysteine in 10 mM HCl, and 200 μL 5 mM o-phthalaldehyde in EtOH, let stand for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18 end-capped
Mobile phase: MeOH:50 mM pH 6.5 phosphate buffer 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 335

CHROMATOGRAM
Retention time: k' 3.5 (α = 1.13, R_s = 1.04)

KEY WORDS
derivatization; chiral; comparison with capillary electrophoresis; comparison with other derivatizing reagents

REFERENCE
Leroy,P.; Bellucci,L.; Nicolas,A. Chiral derivatization for separation of racemic amino and thiol drugs by liquid chromatography and capillary electrophoresis, *Chirality*, **1995**, 7, 235–242.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Solution + 500 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water + 500 μL buffer, let stand for 10 min, extract three times with 2 mL aliquots of n-hexane:ethyl acetate 50:50. Combine the organic layers and evaporate them to dryness at 80°, reconstitute with 2 mL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: solutions, urine
Sample preparation: Add 20 μL of a solution of amphetamine in MeCN:water 50:50 to a cartridge containing 30 mg reagent, heat at 60° for 5 min, elute with 500 μL MeCN, inject a 20 μL aliquot of the eluate. Alternatively, inject 10 μL of urine (adjusted to pH 10 with 100 mM NaOH and filtered) into a 27 × 3 reactor filled with reagent held at 60°, after 5 min elute the contents of the reactor on to the column with mobile phase, elute the column with mobile phase, monitor the effluent from the column. (Prepare reagent as follows. Add 3 g aluminum trichloride to 5 g 60-90 μm macroporous polystyrene-divinylbenzene

copolymer (Fluka) and 10 g 4-chloro-3-nitrobenzoyl chloride stirred in 100 mL dry nitrobenzene, stir at 60° for 5 h, pour into a mixture of 75 mL DMF, 50 mL concentrated HCl, and 50 g ice, filter. Wash the solid with three 50 mL portions of DMF:water 75:25, wash with 30 mL warm (60°) DMF, wash with five 50 mL portions of dichloromethane:MeOH 2:1. Stir the product in 15 mL 40% benzyltrimethylammonium hydroxide in water, 15 mL water, and 30 mL dioxane (Caution! Dioxane is a carcinogen!) at 90° for 8 h, filter. Wash the product with four 50 mL portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with three 50 mL portions of dioxane until the washings are neutral, wash with four 50 mL portions of dichloromethane:MeOH 2:1. Stir 1 g of the yellow product in 10 mL dichloromethane and 500 μL pyridine, add 1.24 g 9-fluorenylmethyl chloroformate, stir at room temperature for 30 min, filter. Wash the solid with three 20 mL portions of DMF, three 20 mL portions of dichloromethane, and three 20 mL portions of dry diethyl ether, dry under vacuum to give the reagent (polymer bound o-nitrobenzophenone fluorenylmethyl carbonate) Store in the freezer, good for at least a year.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChroSpher C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.5
Injection volume: 20
Detector: F ex 265 em 320

CHROMATOGRAM

Retention time: 5
Limit of detection: 100 ppb

OTHER SUBSTANCES

Also analyzed: butylamine, diethylamine, morpholine, propylamine

KEY WORDS

derivatization

REFERENCE

Gao,C.-X.; Chou,T.-Y.; Krull,I.S. Polymeric activated ester reagents for off-line and on-line derivatizations of amine nucleophiles in high-performance liquid chromatography with ultraviolet and fluorescence detection, *Anal.Chem.*, **1989**, *61*, 1538–1548.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 136

CHROMATOGRAM

Retention time: 14.7

Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES

Extracted: 6-acetylmorphine, benzoylecgonine, cocaine, ephedrine, methamphetamine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS

SPE

REFERENCE

Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.5

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam

Interfering: phenylpropanolamine, phentermine, phenmetrazine, lidocaine, ephedrine, pentazocine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of urine to 10 with 1 M NaOH, inject a 10 μL aliquot onto column A heated to 60°, after 6 s stop the flow through column A, after 5 min backflush the contents of column A onto column B with mobile phase, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 27 × 30 packed with polymeric reagent; B 250 × 4 5 μm LiChrospher C18 (Prepare polymeric reagent, polymeric FMOC-L-proline, as follows. Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g 60-90 μm 96% styrene-4% divinylbenzene resin (Fluka) and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Dissolve 600 mg N-(9-fluorenylmethoxycarbonyl)-L-proline in 10 mL dichloromethane, cool to 0°, add 1.8 mmoles dicyclohexylcarbodiimide, stir at 0° for 30 min, filter. Add the filtrate to 1 g resin, add 500 μL pyridine, stir at room temperature for 1 h, filter, wash with three 50 mL portions of hexane, wash with three 50 mL portions of dichloromethane, wash with three 100 mL portions of MeCN.)
Mobile phase: MeCN:water 48:52
Flow rate: 1.5
Injection volume: 10
Detector: UV 265, F ex 265 em 315

CHROMATOGRAM
Retention time: 28 (d), 30 (l)
Limit of detection: 50 ng/mL

KEY WORDS
derivatization; chiral; column-switching

REFERENCE
Gao,C.-X.; Krull,I.S. Determination of enantiomeric drugs in physiological fluids using on-line solid phase derivatizations and reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1183–1198.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 100 μL 25 μg/mL N-n-propylaniline + 6 mL pH 10.0 carbonate buffer + 15 mL water, add mixture to an Extrelut SPE cartridge, let stand for 20 min, elute with 40 mL hexane:ethyl acetate 90:10. Add the eluate to 3 mL 100 mM sulfuric acid and 500 mg NaCl, stir for 20 min, centrifuge at 1000 g for 5 min. Remove the lower layer and add it to 3 mL 2.5 M NaOH and 20 μL benzoyl chloride, stir vigorously

for 30 min. Extract the mixture with 1.5 mL chloroform. Wash the chloroform layer twice with 5 mL water and evaporate it to dryness at 40°, reconstitute the residue in 200 μL hexane:isopropanol 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: Hexane:isopropanol 90:10
Column temperature: 48
Flow rate: 1-1.4
Detector: UV 220

CHROMATOGRAM
Retention time: 17 (l), 25 (d)
Internal standard: N-n-propylaniline (11)
Limit of detection: 25 ng

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Assay of the optical isomers of methamphetamine and amphetamine in rat urine using high-performance liquid chromatography with chiral cellulose-based columns, *J.Chromatogr.*, **1990**, *525*, 203–209.

SAMPLE
Matrix: urine
Sample preparation: Mix 100 μL urine with 60 mg reagent, after 2 min elute with 500 μL MeCN, add 500 μL 50 mM NaOH to the eluate, mix, inject a 20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl: dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb C18
Mobile phase: MeCN:water 50:50 containing 0.05% ammonium hydroxide
Flow rate: 2
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: 4.1

KEY WORDS
derivatization

REFERENCE
Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE
Matrix: urine
Sample preparation: Inject a 10 μL aliquot into a 27 × 2 reactor packed with 65 mg polymeric reagents A and B in a 5:1 ratio heated at 60°, after 10 min flush the contents of the reactor onto the column with the mobile phase. (Prepare the polymeric reagents as follows. Stir mechanically 5 g 60-90 μm polystyrene-divinylbenzene copolymer (Fluka), 10 g 4-chloro-3-nitrobenzoyl chloride, and 3 g aluminum trichloride in 100 mL dry nitrobenzene at 60° for 5 h, pour into a mixture of 75 mL DMF, 50 mL concentrated HCl, and 50 g ice, filter. Wash the solid with three 50 mL portions of DMF:water 75:25, wash with warm (60°) DMF, wash with three 50 mL portions of dichloromethane:MeOH 2:1. Add the solid to 15 mL 40% benzyltrimethylammonium hydroxide in water, 15 mL water, and 30 mL dioxane (Caution! Dioxane is a carcinogen!), heat at 90° for 8 h, filter, wash with four 50 mL portions of warm (60°) dioxane. Add 30 mL acetic acid:water 50:50 and stir for 15 min, filter, wash with water until the washings are neutral, wash the three 50 mL portions of dioxane, wash with four 50 mL portions of dichloromethane:MeOH 2:1. Stir 1.12 g of this product with 248 mg 9-fluorenylmethyl chloroformate in 2 mL dichloromethane and 100 μL pyridine at room temperature for 30 min, filter, wash with three 4 mL portions of DMF, wash with three 4 mL portions of dichloromethane, wash with three 4 mL portions of dry ethyl ether, dry under vacuum to give polymer-bound fluorenylmethoxycarbonyl reagent (A). Substitute 4-nitrobenzoyl chloride for 9-fluorenylmethyl chloroformate to obtain polymer-bound 4-nitrobenzoyl reagent (B).)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher C18
Mobile phase: MeCN:water 55:45
Flow rate: 1.5 for 6 min then 2.5
Injection volume: 10
Detector: UV 265, F ex 265 em 320

CHROMATOGRAM
Retention time: 6 (4-nitrobenzoyl derivative), 15 (fluorenylmethoxycarbonyl derivative)
Limit of quantitation: 10 ppm (F)

KEY WORDS
derivatization

REFERENCE
Gao,C.X.; Schmalzing,D.; Krull,I.S. A mixed-bed, multi-derivatization approach using polymeric reagents for derivatizations of amines in high performance liquid chromatographic detection, *Biomed.Chromatogr.*, **1991**, *5*, 23–31.

SAMPLE
Matrix: urine
Sample preparation: 200-500 μL Rat urine + 200-500 μL pH 3.8 acetate buffer + 25 μL 40 μg/mL β-glucuronidase and 20 μg/mL arylsulfatase (Merck), heat at 37° for 24 h, add 100 μL 25 μg/mL 3-methoxytyramine in water, add 100 μL water, adjust pH to 9.0 with 1.9 M sodium carbonate, add to an Extrelut SPE cartridge, let stand for 20 min, elute with 6 mL ethyl acetate. Add the eluate to 1 mL 100 mM sulfuric acid, extract. Add the aqueous layer to 3 mL 2.5 M NaOH, add 25 μL benzoyl chloride, extract with 5 mL ethyl

acetate. Wash the ethyl acetate layer with water, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL EtOH, 3.5 mL 50 mM pH 8.0 Tris/HCl buffer, and 35 μL esterase (Type 1 porcine liver, Sigma). Heat at 25° for 45 min, add to an activated Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 5 mL acetone. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL hexane:EtOH 89:11, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: n-Hexane:EtOH 89:11
Column temperature: 48
Flow rate: 1.4
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 12 (L), 14 (D)
Internal standard: 3-methoxytyramine (54)

OTHER SUBSTANCES
Extracted: metabolites, methamphetamine

KEY WORDS
rat; SPE; derivatization

REFERENCE
Nagai,T.; Kamiyama,S. Simultaneous HPLC analysis of optical isomers of methamphetamine and its metabolites, and stereoselective metabolism of racemic methamphetamine in rat urine, *J.Anal.Toxicol.*, **1991**, *15*, 299–304.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 3 mL urine to 11 with 10 M KOH, add to an Extrelut 3 column, let stand for 10 min, elute with 15 mL n-hexane into a tube containing one drop of 3 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Add 1.5 mL 8% sodium bicarbonate in water and 1 mL 0.5% sodium naphthoquinone-4-sulfonate in water to the residue, heat at 70° for 20 min, cool, extract with 5 mL carbon tetrachloride. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: MeCN:buffer 55:45 (Buffer was 1.361 g KH_2PO_4 in 950 mL, add 1.3 mL methanesulfonic acid, adjust pH to 3 with 5 M KOH, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 460

CHROMATOGRAM
Retention time: 7.3
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone,

naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE; derivatization

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Adsorbex SCX cation-exchange SPE cartridge (Merck) with 2 mL MeOH, 1 mL water, and 1 mL 17 mM KH_2PO_4, do not allow to dry. Centrifuge urine at 2000 g for 5 min. 1 mL Urine + 500 μL 50 mM KH_2PO_4, sonicate for 1 min, add to the SPE cartridge, rinse vial with 50 μL 50 mM KH_2PO_4 and add to cartridge, dry cartridge for 1 min, wash with three 500 μL portions of 17 mM KH_2PO_4, wash with 1 mL MeOH, dry under vacuum for 1 min, elute with four 500 μL portions of MeOH: 7.3% HCl (97.5:2.5) at a flow rate of 0.5 mL/min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL (8.5 g) 85% orthophosphoric acid and 280 μL (0.22 g) hexylamine per liter. B was MeCN containing 100 mL water, 5 mL (8.5 g) 85% orthophosphoric acid, and 280 μL (0.22 g) hexylamine per liter. A:B 94.5:5.5 for 10.6 min, then to 61:39 over 11 min.
Column temperature: 40
Flow rate: 0.8
Injection volume: 10
Detector: UV 198

CHROMATOGRAM
Retention time: 6
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: 3,4-methylenedioxyamphetamine, methamphetamine, 4-methoxyamphetamine, phentermine, 3,4-methylenedioxymethamphetamine, 5-methoxy-3,4-methylenedioxyamphetamine, 3,4,5-trimethoxyamphetamine, 3,4-methylenedioxyethylamphetamine, 2,5-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyphenylethylamine, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-ethylamphetamine, mescaline, methoxamine

KEY WORDS
SPE

REFERENCE
Helmlin,H.-J.; Brenneisen,R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1992**, *593*, 87–94.

SAMPLE
Matrix: urine
Sample preparation: Condition a 130 mg Bond Elut Certify SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 6.0 phosphate buffer, do not allow to go dry. 2 mL Urine +

800 μL 100 mM pH 6.0 phosphate buffer + 200 μL 10 μg/mL 1-methyl-3-phenylpropylamine in MeOH, if necessary adjust pH to 5-7 with 1 M KOH or 1 M HCl (with pH paper), add the mixture to the SPE cartridge, wash with 1 mL 1 M acetic acid, wash with 3 mL water, dry for 5 min under vacuum, wash with 6 mL MeOH, dry for 2 min under vacuum, elute with 2 mL ethyl acetate:30% ammonium hydroxide 98:2 at a flow rate of 4-6 drops/sec. Evaporate the eluate for 2 min under a stream of nitrogen, add 100 μL 1 M HCl in diethyl ether, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 125 μL MeCN:water:triethylamine 90:8.6:1.4. Add the entire volume to 50 ± 5 mg reagent in a 1 mL plastic pipette tip plugged with a Kimwipe, react for 30 s, elute with 500 μL MeCN under pressure, inject a 10-20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Guard column: 20 mm long Microsorb octadecyldimethylsilyl silica (Rainin)
Column: 10 (sic)x 4.6 5 μm Microsorb octadecyldimethylsilyl silica (Rainin)
Mobile phase: MeCN:10 mM pH 2.5 phosphate buffer 45:55
Flow rate: 0.7
Injection volume: 10-20
Detector: UV 220

CHROMATOGRAM

Retention time: 16.3
Internal standard: 1-methyl-3-phenylpropylamine (23.0)
Limit of detection: 14 ng/mL
Limit of quantitation: 47 ng/mL

OTHER SUBSTANCES

Noninterfering: benzoylecgonine, cocaine, codeine, glutethimide, imipramine, meperidine, methadone, methamphetamine, methaqualone, morphine, nortriptyline, oxazepam, phencyclidine, propoxyphene, quinine

KEY WORDS

SPE; derivatization

REFERENCE

Fisher,D.H.; Bourque,A.J. Quantification of amphetamine in urine: solid-phase extraction, polymeric reagent derivatization and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 142–147.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 75:25 over 7 min, hold at 75:25 for 3 min, return to 10:90 over 5 min, equilibrate at 10:90 for 5 min
Flow rate: 1.5
Injection volume: 20
Detector: UV 190-370

CHROMATOGRAM

Retention time: 4.9

OTHER SUBSTANCES

Extracted: amitriptyline, morphine, codeine, benzoylecgonine, meperidine, norpropoxyphene, nordiazepam
Also analyzed: phenylpropanolamine, lidocaine, diphenhydramine, nortriptyline, ephedrine, cocaine (different gradient).

REFERENCE

Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE

Matrix: urine
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 500 μL MeOH and 500 μL water. Adjust pH of urine to 10, centrifuge at 1500 g. 2 mL Supernatant + 100 μL 75 μg/mL β-phenylethylamine hydrochloride in water, add to the SPE cartridge, wash with 2.5 mL water, elute with 2 mL MeOH, evaporate the eluate to dryness. Reconstitute in water, add 500 μL 8% sodium bicarbonate, add 500 μL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, make up to 1.5 mL with water, heat at 70° for 20 min, cool, add an equal volume of chloroform, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and dry it over anhydrous sodium sulfate, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher Si-60
Mobile phase: EtOH:chloroform:ethyl acetate:n-hexane 1:22:32:45
Flow rate: 2
Injection volume: 25
Detector: UV 280

CHROMATOGRAM

Retention time: 3.7
Internal standard: β-phenylethylamine hydrochloride (4.9)

OTHER SUBSTANCES

Extracted: methamphetamine

KEY WORDS

SPE; normal phase; derivatization

REFERENCE

Campins Falcó,P.; Molins Legua,C.; Herráez Hernandez,R.; Sevillano Cabeza,A. Improved amphetamine and methamphetamine determination in urine by normal-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulphonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *663*, 235–245.

SAMPLE

Matrix: urine

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. 500 μL Urine + 500 μL 2000 U/mL β-glucuronidase with sulfatase activity (Type H-1, Sigma) in 100 mM pH 5 acetate buffer, heat at 37° overnight, add 500 mg NaCl, add 500 μL 50 mM pH 11 potassium phosphate buffer, add 1.3 μg 4'-hydroxymethamphetamine, add 3.1 μg methamphetamine, adjust pH to 11 with ammonium hydroxide, mix. Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. Add the mixture to the SPE cartridge, wash with 1 mL 50 mM pH 11 potassium phosphate buffer, wash with 1 mL freshly prepared MeOH:water 30:70, wash with 1 mL MeCN, elute with 1 mL freshly prepared MeCN: acetic acid 98:2, elute with 1 mL MeCN:HCl 98:2. Combine the eluates and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in mobile phase (?), inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: phenyl
Column: Microsorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 3 potassium phosphate 5:10:85
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM

Retention time: 12.2
Internal standard: 4'-hydroxymethamphetamine (6.0), methamphetamine (15.6)
Limit of quantitation: 920 ng/mL

OTHER SUBSTANCES

Extracted: 4'-hydroxyamphetamine, metabolites

KEY WORDS

SPE; rat

REFERENCE

Law,M.Y.L.; Moody,D.E. Simultaneous quantitation of amphetamine and 4'-hydroxyamphetamine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2029–2043.

SAMPLE

Matrix: urine

Sample preparation: Condition an Extra-Sep C18 SPE cartridge (Teknokroma) with 1 mL MeOH and 1 mL buffer. Adjust pH of 2 mL urine to ca. 10 with 100 μL concentrated ammonium hydroxide, add 5 μg β-phenylethylamine, add to the SPE cartridge, wash with 5 mL water, wash with 1 mL MeCN, elute with 2 mL MeOH. Add 100 μL EtOH:concentrated HCl 6:1 to the eluate, evaporate to dryness. Reconstitute with 1 mL buffer and 1 mL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, let stand at room temperature for 10 min, add 2 mL n-hexane:ethyl acetate 50:50, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 1% aqueous sodium bicarbonate adjusted to pH 10 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: Gradient. MeCN:0.5% propylamine in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 4.7
Internal standard: β-phenylethylamine (4.1)
Limit of detection: 4 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
SPE; derivatization

REFERENCE
Molíns Legua,C.; Campíns Falcó,P.; Sevillano Cabeza,A. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulfonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *672*, 81–88.

SAMPLE
Matrix: urine
Sample preparation: 100-300 μL Urine + 100 μL 1.5 M NaOH + 5 μg IS, make up to 1 mL with water, add to an Extrelut 1 SPE cartridge, let stand for 20 min, elute with 6 mL benzene (Caution! Benzene is a carcinogen!). Add the eluate to 1 mL 100 mM sulfuric acid, extract. Remove the aqueous layer and add it to 3 mL 1.5 M NaOH, add 5 μL benzoyl chloride, vortex vigorously, extract twice with 3 mL portions of n-hexane. Combine the organic layers and wash them twice with 3 mL portions of water, evaporate the organic to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Chiralcel OB-H
Mobile phase: n-Hexane:isopropanol 90:10
Column temperature: 55
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5 (L), 18 (D)
Internal standard: l-p-methoxyamphetamine (12)
Limit of detection: 30 ng

OTHER SUBSTANCES
Extracted: ethylamphetamine, methamphetamine

KEY WORDS
rat; derivatization; SPE; chiral

REFERENCE

Nagai,T.; Kamiyama,S.; Matsushima,K. Analysis of time-lapse changes of d- and l-enantiomers of racemic ethylamphetamine and stereoselective metabolism in rat urine by HPLC determination, *J.Anal.Toxicol.*, **1995**, *19*, 225–228.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)

Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)

Flow rate: A 1.25; B 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 9.2

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

KEY WORDS

column-switching

REFERENCE

Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 20-fold or more. 500 μL Diluted urine + 50 μL 500 ng/mL (+)-2,5-dimethoxyamphetamine hydrochloride + 100 μL 500 mM NaOH + 2 mL benzene (Caution! Benzene is a carcinogen!), shake for 15 min, centrifuge at 1200 g for 5 min. Remove 1.8 mL of the organic phase and add it to 220 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove 200 μL of the aqueous phase and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 3 mM (-)-1-(9-fluorenyl)ethyl chloroformate, let stand at room temperature for 24 h, add 30 μL 100 mM glycine in water, let stand for 30 min, add 750 μL pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a centrifugal evaporator at room temperature, reconstitute the residue in 300 μL MeCN:water 50:50, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Adsorbosphere HS C18

Mobile phase: MeCN:THF:20 mM pH 3.6 sodium acetate buffer 25:21:54

Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 37 (L), 40 (D)
Internal standard: (+)-2,5-dimethoxyamphetamine (33)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
rat; derivatization; chiral

REFERENCE
Sukbuntherng,J.; Hutchaleelaha,A.; Chow,H.-H.; Mayersohn,M. Separation and quantitation of the enantiomers of methamphetamine and its metabolites in urine by HPLC: Precolumn derivatization and fluorescence detection, *J.Anal.Toxicol.*, **1995**, *19*, 139–147.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 1% pH 10 sodium bicarbonate buffer. 2 mL Urine + 400 μL 8% pH 10 sodium bicarbonate buffer, mix, centrifuge at 1500 g for 2 min, add a 2 mL aliquot of the supernatant to the SPE cartridge, wash with 3 mL water, pass 500 μL 2% sodium 1,2-naphthoquinone 4-sulfonate through the cartridge, pass 500 μL 1% pH 10 sodium bicarbonate buffer through the cartridge, let stand at room temperature for 15 min, wash with 3 mL water, elute with 1 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:buffer from 40:60 to 50:50 over 2.5 min, to 70:30 over 0.5 min, maintain at 70:30 for 1.5 min, to 85:15 over 1 min, maintain at 85:15 for 1.5 min. (Buffer was 5 mL/L propylamine in water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.1
Internal standard: β-phenylethylamine (3.6)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
derivatization; SPE

REFERENCE
Campíns-Falcó,P.; Sevillano-Cabeza,A.; Molíns-Legua,C.; Kohlmann,M. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with simultaneous sample clean-up and derivatization with 1,2-naphthoquinone 4-sulphonate on solid-phase cartridges, *J.Chromatogr.B*, **1996**, *687*, 239–246.

SAMPLE
Matrix: urine

Sample preparation: Inject 15 μL urine, inject a mixture of 5 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN and 45 μL water, and inject 10 μL buffer on to column A and elute to waste with mobile phase A. After 2.8 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. At the end of the run condition column A with 1 mL mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 PR-C18
Mobile phase: A water; B Gradient. MeCN:water from 40:60 to 70:30 over 15 min. to 100:0 over 5 min.
Flow rate: A 0.35; B 1
Injection volume: 15
Detector: F ex 264 em 313

CHROMATOGRAM

Retention time: 15.3
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: ephedrine, methamphetamine, norephedrine, 3-phenylpropylamine, pseudoephedrine

KEY WORDS

column-switching; derivatization; on-column derivatization

REFERENCE

Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 69–78.

SAMPLE

Matrix: urine
Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 2 min inject a mixture of 25 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water and 25 μL buffer on to column A, stop the flow of mobile phase A, after 10 min start pump A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. After each run flush column A with ethyl acetate for 1 min, n-hexane for 1 min, and ethyl acetate for 1 min, re-equilibrate with mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS C18
Mobile phase: A water; B Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 5
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: methamphetamine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herréz-Hernńdez,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 20 μL 100 μM 1-phenylethylamine in water + 400 μL concentrated HCl, heat at 80° for 1 h, cool, neutralize with 600 μL 25% ammonia, add 5 mL 10% sodium carbonate solution, add 2 mL 500 mM pH 10.5 sodium borate buffer, add 2 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 12.5° at 1500 g for 10 min, repeat the extraction. Combine the organic layers and remove a 100 μL aliquot, add 10 μL acetic acid to the aliquot, evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL carbonate buffer, add 50 μL 10 mM fluorescein-4-isothiocyanate in EtOH, mix, heat in the dark at 80° for 15 min, inject a 20 μL aliquot. (Prepare carbonate buffer by adjusting the pH of 200 mM sodium bicarbonate to 9.0 with 200 mM sodium carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Daisopak SP-120-5-ODS (Daiso, Osaka)
Mobile phase: Gradient. MeCN:20 mM pH 7.9 sodium phosphate buffer 20:80 for 16 min then 24:76 (step-gradient).
Flow rate: 0.8
Injection volume: 20
Detector: F ex 496 em 518

CHROMATOGRAM
Retention time: 32
Internal standard: 1-phenylethylamine (26.6)
Limit of detection: 5.5 nM

OTHER SUBSTANCES
Extracted: metabolites, methamphetamine, norepinephrine

KEY WORDS
derivatization

REFERENCE
Al-Dirbashi,O.; Kuroda,N.; Akiyama,S.; Nakashima,K. High-performance liquid chromatography of methamphetamine and its related compounds in human urine following derivatization with fluorescein isothiocyanate, *J.Chromatogr.B*, **1997**, *695*, 251–258.